

DATA EVALUATION RECORD

PICOXYSTROBIN (ZA1963)

Study Type: OPPTS 870.3150 [§82-1b]; Subchronic Oral Toxicity Study in Dogs

Work Assignment No. 7-01-256 C (MRID 48073734)

Prepared for
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DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral Toxicity in Dogs (feeding); OPPTS 870.3150 ['82-1b];
OECD 409.

PC CODE: 129200**DP BARCODE:** D378236**TXR #:** 0056696**SUBMISSION:** S873059**TEST MATERIAL (PURITY):** Picoxystrobin (ZA1963; 94.4% a.i.)

SYNONYMS: Methyl (αE)- α -(methoxymethylene)-2-[[[6-(trifluoromethyl)-2-pyridinyl]oxy]
methyl]benzeneacetate

CITATION: Horner, S.A. (1998) ZA1963: 90-day dietary toxicity study in dogs. Central
Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK.
Laboratory Study Report No.: CTL/P/5643, March 25, 1998. MRID
48073734. Unpublished.

SPONSOR: E. I. du Pont de Nemours and Company, Wilmington, DE

EXECUTIVE SUMMARY: In a subchronic oral toxicity study (MRID 48073734), ZA1963
(Picoxystrobin; 94.4% a.i.; Batch No. P27) was administered in the diet to four beagle
dogs/sex/dose group at concentrations of 0, 125, 250, or 500 ppm (equivalent to 0, 4.3/4.3,
8.9/8.5, or 16.5/16.9 mg/kg/day in males/females) for at least 90 days.

No adverse effects of treatment were observed on mortality, clinical signs, ophthalmoscopic
examinations, hematology, clinical chemistry, organ weights, or gross or microscopic pathology.

At 500 ppm, body weights were decreased during Weeks 2-14 by 4-7% ($p \leq 0.05$, except Weeks
13 and 14) in the males and by 3-7% ($p \leq 0.05$, except Week 6) in the females, resulting in
decreased overall body weight gains in both males and females. Additionally at this dose, food
consumption was decreased ($p \leq 0.05$; except during Week 5) during Weeks 1-6 by 7-27% in the
males and by 8-26% in the females.

At 250 ppm and 125 ppm, treatment-related effects were not observed.

**The LOAEL is 500 ppm (16.5 mg/kg/day), based on decreased body weights and body
weight gains in the males and food consumption in males and females. The NOAEL is 250
ppm (8.5 mg/kg/day).**

This study is classified **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.3150; OECD 409) for a subchronic oral toxicity study in dogs.

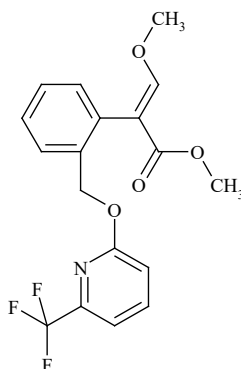
COMPLIANCE: Signed and dated Data Confidentiality, GLP Compliance, and Quality Assurance statements were provided. A Flagging statement was not included.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Description:	Picoxystrobin (ZA1963)
Batch No.:	P27
Purity:	94.4% a.i.
Stability:	Stable in the diet for up to 48 days at room temperature
CAS #:	117428-22-5
Structure:	



2. Vehicle: Diet

3. Test animals

Species:	Dog
Strain:	Beagle
Age and weight at initiation of treatment:	Approximately 20-28 weeks; 7.4-11.0 kg males, 7.0-9.0 kg females
Source:	Animal Breeding Unit, Zeneca Pharmaceuticals (Alderly Park, Macclesfield, Cheshire, UK)
Housing:	The dogs were housed by treatment group, sexes separately, in indoor pens. The pens had a sleeping platform with a heated floor underneath and interlinking gates which enabled the dogs to be separated for feeding.
Diet:	Laboratory Diet A (Special Diet Services Ltd., Witham, Essex, UK) was fed at 350 g/day to males and 300 g/day to females
Water:	Tap water, <i>ad libitum</i>
Environmental conditions	
Temperature:	19±4°C
Humidity:	40-70%
Air changes:	Approximately 15/hour
Photoperiod:	12 h light/12 h dark
Acclimation period:	Approximately 4 weeks

B. STUDY DESIGN

1. In life dates: Start: February 4, 19/97 End: Approximately May 4, 1997

2. **Animal assignment:** The dogs were allocated to treatment groups by means of a randomization procedure based on body weight which ensured that litter mates were in different groups, as shown in Table 1. The study was divided into two randomized blocks comprising either four male or four female replicates. Each replicate consisted of four dogs, one per treatment group.

TABLE 1: Study design ^a				
Test group	Dose group (ppm) ^b	Actual dose (mg/kg/day) M/F	# Males	# Females
Control	0	0/0	4	4
Low dose	125	4.3/4.3	4	4
Mid dose	250	8.9/8.5	4	4
High dose	500	16.5/16.9	4	4

a Data were obtained from page 18 and Table 4 on page 37 of the study report.

b Dietary formulations were adjusted for the purity of the test compound.

3. **Dose-selection rationale:** It was stated that the doses used for this study were selected based on the results of a preliminary oral dose range-finding study that used one dog/sex/dose, and that this study was carried out by the Performing Laboratory. No additional information was provided.
4. **Dose preparation, administration, and analysis:** The dietary formulations were prepared by mixing appropriate amounts of the test compound (adjusted for purity) with the basal diet to form premixes. The premixes were then blended with additional basal diet to yield the desired dietary concentrations. Water was added at a rate of approximately 100 mL/kg of dietary formulation and mixed thoroughly. The dietary formulations were then pelleted, and the pellets dried by exposing them to the residual heat of an autoclave (approximately 56°C for 1 hour 45 minutes). The dried pellets were then stored in a bin at room temperature. Homogeneity (top, middle, bottom) and stability analyses were performed on the 125 and 500 ppm formulations of the first batch. Stability analyses were performed on dietary formulations stored at room temperature for up to 48 days. Concentration analyses were performed on all dose levels of the first batch and at approximately the mid-point of the dosing period, and on both powdered and pelleted formulations at all dose levels of the first batch.

Results

Homogeneity (%CV): 0.49-4.71%

Stability (% of initial): 96.7-101.9% following room temperature storage for 48 days

Concentration (% of nominal): 89.4-100.0% for pelleted dietary formulations

Powdered dietary formulations had concentrations of 97.4-104.0% of nominal, while pelleted formulations had concentrations of 89.4-95.6% of nominal, indicating that the test compound was stable in the presence of water and heat. The analytical data indicated that the mixing procedure was adequate and that the variation between nominal and actual dosage to the animals was acceptable.

5. **Statistics:** Significance was reported at $p \leq 0.05$ and $p \leq 0.01$. Body weights were subjected to analysis of covariance (ANCOVA) on Week 1 body weights, separately for males and females. Weekly food consumption was subjected to analysis of variance (ANOVA), separately for males and females. Hematology and clinical chemistry were subjected to ANCOVA on pre-experimental values. Male and female values were analyzed together; the results were examined to determine if any differences between control and treated groups were consistent between sexes. The covariate adjustment was based on the separate sex pre-experimental group means. Organ weights were analyzed by ANOVA and ANCOVA on terminal body weights, separately for male and female. The data from paired organs were examined for differential effects on left and right components.

ANOVA and ANCOVA, with the exception of organ weights, allowed for the replicate structure of the study design. Differences from the controls were tested statistically by comparing each treatment group least-squares mean with the control group least-squares mean using a two-sided Student's t-test, based on the error mean square in the analysis. The statistical analyses were considered appropriate.

C. **METHODS**

1. **Observations**

- a. **Clinical observations:** The dogs were observed at least three times daily (at dosing, after dosing, and at the end of the work day) for clinical abnormalities. An individual daily assessment of fecal consistency was performed for up to 5 hours post-dosing for all dogs. A detailed physical examination of each dog was performed weekly, and a full clinical examination by a veterinarian was performed prior to study initiation and prior to termination.
- b. **Neurological evaluations:** Neurological examinations were not conducted.
2. **Body weight:** All dogs were weighed prior to study initiation, on Day 1, and then weekly until termination. The dogs were weighed before the diets were provided.
3. **Food consumption:** Each morning, 350 gm and 300 gm of prepared diets were given to the test males and females, respectively. Food consumption was measured daily approximately 4 hours after feeding. Food consumption (g/dog/day) was reported weekly during the treatment period.
4. **Ophthalmoscopic examination:** An indirect ophthalmoscopic examination was performed on all dogs prior to study initiation and prior to termination.

5. **Hematology and clinical chemistry:** Blood samples were collected from the jugular vein of all dogs prior to feeding on Weeks -1, 4, 8 and prior to termination. The following CHECKED (X) parameters were examined.

a. **Hematology**

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpuscular HGB concentration (MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpuscular volume (MCV)*
X	Platelet count*		Reticulocyte count
	Blood clotting measurements*	X	Blood cell morphology
	(Activated partial thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Recommended for 90-day oral non-rodent studies based on Guideline 870.3150

b. **Clinical chemistry**

	ELECTROLYTES		OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Creatinine*
	Magnesium	X	Urea nitrogen*
X	Phosphorus*	X	Total cholesterol*
X	Potassium*		Globulins
X	Sodium*	X	Glucose*
	ENZYMES (more than 2 hepatic enzymes eg.,*)	X	Total bilirubin*
X	Alkaline phosphatase (ALP)*	X	Total protein (TP)*
	Cholinesterase (ChE)		Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)		Albumin/globulin ratio
X	Alanine aminotransferase (ALT; SGPT)*		
X	Aspartate aminotransferase (AST; SGOT)*		
	Sorbitol dehydrogenase*		
X	Gamma glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		

* Recommended for 90-day oral non-rodent studies based on Guideline 870.3150

6. **Urinalysis:** Urinalysis was not performed.

7. **Sacrifice and pathology:** All dogs were euthanized by exsanguination while under deep sodium pentobarbitone anesthesia, and subjected to a gross necropsy. The CHECKED (X) tissues were collected for microscopic examination. Additionally, the (XX) organs were weighed from all animals. Paired organs were weighed separately.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue	X	Aorta, abdominal*	XX	Brain* +
X	Salivary glands*	X	Heart* +	X	Peripheral nerve (sciatic)*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	X	Spleen* +	X	Eyes (with optic nerves)*
X	Jejunum*	X	Thymus* +		GLANDULAR
X	Ileum*			XX	Adrenal gland* +
X	Cecum*		UROGENITAL		Lacrimal gland
X	Colon*	XX	Kidneys* +	X	Parathyroid* +
X	Rectum*	X	Urinary bladder*	XX	Thyroid* +
XX	Liver* +	XX	Testes* +		OTHER
X	Gall bladder* +	X	Epididymides* +	X	Bone (femur, stifle joint, sternum)
X	Pancreas*	X	Prostate*	X	Skeletal muscle
	RESPIRATORY	XX	Ovaries* +	X	Skin*
X	Trachea*	X	Uterus* +	X	All gross lesions and masses*
X	Lungs*	X	Mammary gland (females only)*		
	Nasal cavity*	X	Cervix (with vagina)		
	Pharynx*	X	Oviduct		
	Larynx*				

* Recommended for 90-day oral non-rodent studies based on Guideline 870.3150

+ Organ weight required for non-rodent studies.

All tissues were preserved in “an appropriate fixative.” The femur and stifle, epididymis, mammary gland, prostate, skin, trachea, and skeletal muscle were not processed for examination. Tissue samples were processed routinely and stained with hematoxylin and eosin. All tissues and gross lesions and masses from all dogs were examined microscopically. Microscopic pathological findings were graded as minimal, slight, moderate, or marked.

II. RESULTS

A. OBSERVATIONS

- Mortality:** All dogs survived to the scheduled termination.
- Clinical signs of toxicity:** There were no adverse treatment-related clinical observations. An increased incidence of salivation at dosing was noted in the 500 ppm females (24 observations during Weeks 2-13) compared to the controls (4 observations during Weeks 8-13). In the males, group observations of fluid feces were recorded a total of 5 times at 250 ppm and 18 times at 500 ppm compared to 0 times in the controls. In the females, group observations of fluid feces were recorded a total of 6 times at 500 ppm compared to 1 time in the controls. The fluid feces was not observed in the 1-year study (MRID 48073741), and the toxicological significance was not clear. All other clinical observations were transient, occurred in a single animal, and/or were unrelated to dose.

- B. BODY WEIGHT AND WEIGHT GAIN:** Selected body weight and body weight gain data are presented in Table 2. In the 250 ppm males, body weights were slightly decreased, but the decrease did not statistical significance. The decrease in 250 ppm was not treatment related. At 500 ppm, body weights were decreased during Weeks 2-14 by 4-7% ($p \leq 0.05$, except Weeks 13 and 14) in the males and by 3-7% ($p \leq 0.05$, except Week 6) in the females, resulting in a decreased overall body weight gains ($\downarrow 38\%$ in males and $\downarrow 45$ in females).

Examining the individual animal data, the mean body weight decrease in high dose males appeared to be driven by a single dog. In a study where reasonable number of test animals are used (10 or more), the effects driven by a single test animals will not be considered treatment-related. However for this study, the number of experimental animal employed is small (4), and the effect of a single animal can't be ignored as demonstrated by the results of the statistical analysis.

TABLE 2. Selected group mean (\pm SD) body weights and body weight gains (kg) in dogs administered ZA1963 in the diet for up to 13 weeks ^a				
Study Week	Dose (ppm)			
	0	125	250	500
Males				
1	9.23 \pm 0.93	9.18 \pm 1.15	9.10 \pm 0.88	9.20 \pm 1.47
2	9.38 \pm 0.78	9.30 \pm 1.02	9.15 \pm 0.79	8.98 \pm 1.43
Adjusted Mean ^c	9.32	9.30	9.23	8.95** ($\downarrow 4$)
3	9.60 \pm 0.77	9.43 \pm 0.99	9.33 \pm 0.92	9.20 \pm 1.47
Adjusted Mean	9.55	9.43	9.40	9.17** ($\downarrow 4$)
9	10.65 \pm 0.78	10.45 \pm 0.90	10.00 \pm 1.09	10.03 \pm 1.48
Adjusted Mean	10.59	10.45	10.08	10.00* ($\downarrow 6$)
12	11.13 \pm 0.83	10.85 \pm 0.90	10.45 \pm 1.07	10.33 \pm 1.57
Adjusted Mean	11.07	10.85	10.54	10.30** ($\downarrow 7$)
14	11.20 \pm 0.88	11.05 \pm 0.87	10.58 \pm 1.07	10.45 \pm 1.61
Adjusted Mean	11.14	11.05	10.66	10.42 ($\downarrow 6$)
BWG Weeks 1-14 ^b	1.97	1.87	1.56	1.22 ($\downarrow 38\%$)
Females				
1	7.95 \pm 0.88	8.05 \pm 0.62	7.95 \pm 0.70	7.83 \pm 0.61
2	8.08 \pm 0.85	8.13 \pm 0.54	8.00 \pm 0.76	7.73 \pm 0.67
Adjusted Mean	8.07	8.00	7.99	7.86* ($\downarrow 3$)
11	9.23 \pm 0.75	9.03 \pm 0.37	9.03 \pm 0.49	8.45 \pm 0.52
Adjusted Mean	9.22	8.88	9.02	8.61* ($\downarrow 7$)
14	9.43 \pm 0.83	9.45 \pm 0.33	9.35 \pm 0.48	8.65 \pm 0.44
Adjusted Mean	9.42	9.30	9.34	8.82* ($\downarrow 6$)
BWG Weeks 1-14 ^b	1.48	1.40	1.40	0.82 ($\downarrow 45$)

a Data were obtained from Table 9 on pages 49-52 of the study report. Percent differences from controls (calculated by reviewers) are included in parentheses. n=4

b Calculated by reviewers from data (unadjusted means) presented in this table.

c These values were adjusted for intergroup differences in group mean initial body weight.

* Significantly different from controls; $p \leq 0.05$

** Significantly different from controls; $p \leq 0.01$

- C. FOOD CONSUMPTION:** Selected food consumption data are presented in Table 3. At 500 ppm, food consumption was decreased ($p \leq 0.05$; except during Week 5) during Weeks 1-6 by 7-27% in the males and by 8-26% in the females. Thereafter, decreases in food consumption were minor ($< 5\%$ in the males; $< 7\%$ in females) and and/or did not attain statistical significance. In the 250 ppm males, food consumption was decreased by 9%

during Week 1 and reported to be statistically different from the controls. However, this decrease was not considered adverse because it was slight and not seen after Week 1. In addition, no food consumption effect was seen in 250 ppm females.

Food consumption was unaffected by treatment in 150 ppm males and females and in the 250 ppm females.

TABLE 3. Selected group mean (\pm SD) food consumption (g/dog/day) in dogs administered ZA1963 in the diet for up to 13 weeks ^a				
Study Day	Dose (ppm)			
	0	125	250	500
Males				
1	350 \pm 0	339 \pm 21	318 \pm 23* (\downarrow 9)	256 \pm 26** (\downarrow 27)
4	350 \pm 0	350 \pm 0	350 \pm 0	324 \pm 14** (\downarrow 7)
6	350 \pm 0	350 \pm 0	350 \pm 0	311 \pm 21** (\downarrow 11)
13	350 \pm 0	350 \pm 0	350 \pm 0	331 \pm 35
Females				
1	300 \pm 0	295 \pm 10	264 \pm 36	221 \pm 42** (\downarrow 26)
4	300 \pm 0	300 \pm 0	300 \pm 0	275 \pm 14** (\downarrow 8)
6	300 \pm 0	300 \pm 0	300 \pm 0	269 \pm 12** (\downarrow 10)
13	300 \pm 0	300 \pm 0	300 \pm 0	294 \pm 12

a Data were obtained from Table 10 on pages 53-56 of the study report. Percent differences from controls (calculated by reviewers) are included in parentheses. n=4

* Significantly different from controls; $p \leq 0.05$

** Significantly different from controls; $p \leq 0.01$

D. **COMPOUND INTAKE:** The actual dosages of the test material to the dogs are presented in Table 1.

E. **OPHTHALMOSCOPIC EXAMINATION:** There were no treatment-related findings observed during the ophthalmoscopic examinations.

F. **BLOOD ANALYSES**

1. **Hematology:** Platelet count was slightly increased at Weeks 4, 8, and 13 in the 250 ppm males and in 500 ppm males relative to the controls (Table 4). When comparing to the pretreatment values the increase was minimal. Although there were reported to show statistical significance, in the absence of other related hematological changes and corroborating findings of toxicity and dose-related response, these slight changes were not considered adverse or to be treatment-related.
2. **Clinical chemistry:** At 500 ppm, plasma albumin was slightly decreased by 6-10% in the males and females, and total protein was decreased by 4-9% in the males and females. In the absence of corroborating findings of toxicity, slight changes in these two parameters were not considered adverse or treatment related.

Table 4. Mean Platelet Counts in Picoxystrobin Treated males (x10 ⁹ /l)					
Week in Study		Dietary Concentration (ppm)			
		0	100	250	500
Pre-treatment	Mean (SD)	389 ± 79	305 ± 128	374 ± 102	381 ± 63
Week 4	Mean (SD)	317 ± 67	309 ± 62	382 ± 118	376 ± 63
	Adjusted Mean ⁺	298	348	373**	363**
Week 8	Mean (SD)	314 ± 58	320 ± 65	381 ± 102	397 ± 45
	Adjusted Mean	296	356	373*	384**
Week 13	Mean (SD)	335 ± 23	336 ± 49	365 ± 89	399 ± 40
	Adjusted Mean	319	369	358	388 **

Data excerpted from the report , page 63.

⁺: Adjusted mean was reported to be calculated statistically using analysis of covariance.

*: Statistically significant (p<0.05); **: Statistically significant (p<0.01).

G. URINALYSIS: Urinalysis was not performed.

H. SACRIFICE AND PATHOLOGY

- 1. Organ weight:** There were no adverse, treatment-related effects observed on organ weights. In the 500 ppm males, kidney weight (adjusted for body weight) was increased (NS) by 12% for combined left and right organs. The left kidney weight (adjusted for body weight) was increased (NS) by 7%, and the right kidney weight (adjusted for body weight) was increased (p≤0.05) by 17%. In the absence of corroborating microscopic findings, these increases were not considered adverse. No other differences (p≤0.05) in organ weights were observed.
- 2. Gross pathology:** There were no adverse, treatment-related effects observed at necropsy. All gross pathological findings were observed in a single animal, were not dose dependent, and/or were not considered adverse.
- 3. Microscopic pathology:** There were no adverse, treatment-related microscopic changes observed in any of the tissues or organs examined. Minimal focal unilateral tubular dilatation with eosinophilic casts was observed in the kidney in 3/4 of the 500 ppm females. This minimal finding was not considered adverse. All other microscopic observations were observed in a single dog, were of minimal severity, and/or were unrelated to dose.

III. DISCUSSION and CONCLUSIONS

- A. INVESTIGATORS' CONCLUSIONS:** Dietary administration of ZA1963 at 500 ppm produced reduced growth, reduced food consumption, and slight reductions in plasma albumin and/or total protein levels in males and/or females. Reduced growth, without any adverse effects on food consumption, was also seen for males receiving 250 ppm ZA1963.
- B. REVIEWERS' COMMENTS:** No adverse effects of treatment were observed on mortality, clinical signs, ophthalmoscopic examinations, hematology, clinical chemistry, organ weights, or gross or microscopic pathology.

At 500 ppm, body weights were decreased during Weeks 2-14 by 4-7% ($p \leq 0.05$, except Weeks 13 and 14) in the males and by 3-7% ($p \leq 0.05$, except Week 6) in the females, resulting in decreased overall body weight gains in both males and females. Additionally at this dose, food consumption was decreased ($p \leq 0.05$; except during Week 5) during Weeks 1-6 by 7-27% in the males and by 8-26% in the females.

At 250 ppm and 125 ppm, treatment-related effects were not observed.

The LOAEL is 500 ppm (16.5 mg/kg/day), based on decreased body weights and body weight gains in the males and food consumption in males and females. The NOAEL is 250 ppm (8.5 mg/kg/day).

This study is classified **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.3150; OECD 409) for a subchronic oral toxicity study in dogs.

C. **STUDY DEFICIENCIES:** The following minor deficiencies were noted but do not affect the conclusions of this review:

- Blood clotting measurements were not performed.
- Urinalysis was not performed.
- The gall bladder, heart, spleen, thymus, epididymides, uterus, and parathyroids were not weighed.
- The nasal cavity, pharynx, and larynx were not collected.